Asian Journal of Chemistry; Vol. 27, No. 11 (2015), 4022-4028

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.19061

Effect of Cadmium on Antioxidase System in Process of Maize Germination

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Received: 27 January 2015; *Accepted*: 11 March 2015; *Published online*: 16 July 2015; AJC-17394

In order to know something about toxicity mechanism of heavy metals on plants and to improve tolerance of plants to heavy metal poison, the changes of oxidative metabolism and the molecular mechanism in plants under heavy metal stress were explored in the paper. In this test, the maize seeding that has certain tolerant ability to cadmium was used as material. After the maize seeding was treated by different Cd^{2+} concentrations varied from 5 to 100 μmol L^{-1} for 12 h, reactive oxygen metabolism of the seeding was comprehensively investigated and antioxidant enzyme activities with different dose of cadmium treating the seeding was analyzed based on the latest structural information about antioxidant enzyme reported. From the latest structural information about enzymes in the Protein Data Bank (PDB), it is indicated that during germination of cadmium-tolerant maize, antioxidant enzyme system (SOD, POD, CAT, APX) has been found to act effectively as oxygen free radical scavenger in a certain range of Cd^{2+} concentration, while Cd^{2+} concentration goes slightly beyond the threshold most antioxidant enzymes are easy to loss activities, because malondialdehyde is the production of lipid peroxide inside cells, its higher content reflects that plasma membrane is severely damaged. Moreover, content of active oxygen such as hydrogen peroxide, superoxide anion, *etc*. produced in metabolic processes has a higher level, which is highly toxic to the organism especially the plasma membrane. Therefore, it can be concluded that there are significant differences in the different activities of antioxidant enzymes under different conditions. Those experimental results have also verified the information about the molecular structures of 4 antioxidant enzymes given above.

Keywords: Heavy metal stress, Cadmium dose, Antioxidant enzyme system, Enzymatic activity, Maize seeding.

INTRODUCTION

Environmental pollution caused by heavy metal has seriously endangered human health. Cadmium is one of the most toxic pollutants discovered in the air, water and soil, but a non-essential element for the plants and animals. High level of cadmium can interfere with photosynthesis, restrain respiration and affect on the absorption, transport and metabolism of nutrient elements, influencing the plant growth and development. Simultaneously, cadmium can also induce the generation of reactive oxygen species (ROS), resulting in oxidative stress and disturbance of anti-oxidization system[1,2]. Therefore, exploring the changes of oxidative metabolism and the molecular mechanism in plants under heavy metal stress is the key to understand toxicity mechanism of heavy metals and to improve tolerance of plants to heavy metal poison.

Because cadmium is one of non-reduced heavy metals, it cannot involve in Fenton chemical reactions. However, by interfering with electron transport chain of PSI and PSII reaction pathways and anti-oxidative defense mechanisms, cadmium may induce plants to generate a lot of ROS such as superoxide radical ($O_2^{\bullet}$) and hydrogen peroxide (H_2O_2) that can react with proteins, membrane lipids and DNA base, *etc*., causing cell damage or even death[3-6]. Hence, plants have to adapt to different environments for normal growth. On the one hand, by means of vacuole isolation, chelating agent coordination, cell wall fixation, *etc*., plants may decrease cadmium damage[7]. On the other hand, they can lead to different ROS scavenging mechanisms including enzyme scavenging system and non-enzyme scavenging system, among which both superoxide dismutase (SOD) and catalase (CAT), which play a very important role in protecting organisms from oxygen toxicity, are the most critical components of enzyme scavenging system. Superoxide dismutase includes Cu/Zn-SOD in cytoplasm, nuclei, peroxide, chloroplasts, Fe-SOD, which dose not exist in all plants, in chloroplast and Mn-SOD in mitochondria and peroxide, among which Cu/Zn-SOD is the most abundant. Moreover, the former two is regulated by oxidative stress[8,9]. Superoxide dismutase can turn $O_2^{\bullet}$ into H_2O_2 and O_2 by disproportionate reaction is the first line of defense against scavenging reactive oxygen in an organism. Meanwhile, catalase can decompose H_2O_2 into H_2O and O_2 catalytically. Catalase

primarily existing in the loop body of peroxisome and glyoxylic acid, it can not only clear away H_2O_2 which is produced during the course of photorespiration and β-oxidation of fatty acid but also break down H_2O_2 into H_2O and O_2 without external substrate in peroxide[10].

In recent years, a number of researches have been reported on the generation of ROS and the changes in enzyme activity after ROS scavenging in plants under cadmium stress[4,6,7,11], however, the reports based on the latest structural information of the antioxidant enzymes is still less. In this test, the maize seeding that has certain tolerant ability to cadmium was used as material. After the maize seeding was treated by different cadmium concentrations varied from 5 to 100 μmol L^{-1} for 12 h, ROS metabolism of the seeding was comprehensively studied and antioxidant enzyme activities with different dose of cadmium treating the seed were analyzed. This work will provide important basis for an in-depth understanding of physiological and molecular mechanisms for plants adapting to cadmium toxicity.

EXPERIMENTAL

Absorbance was recorded on a TU-1901 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing) at room temperature. Constant temperature incubator is used to cultivate maize seed at 28 °C. TopPette pipetting guns with different specifications of 0.1~5000 μL were used for taking different volumes of solutions. Fresh samples were ground in a mortar. The maize seed, obtained from China Agricultural University, were hybridization of 31×P138. All chemicals, such as quartz sand, benzene, phenol, guaiacol, hydrogen peroxide, PVP, hydrochloric acid, cadmium chloride, boric acid, acetic acid, ethylene diamine tetra-acetic acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, ascorbic acid, thiobarbituric acid, chloroacetic acid, *etc*., used in this work were of analytical grade. Unless otherwise stated, all the solutions were prepared in double distilled water.

Cultivation of maize seeds: The five batches of selected corn seeds, each group of 30 seeds, soaked with 75 % ethanol after washing with double distilled water and then immersed in double distilled water for 4 h. The corn seeds each were put on the filter paper, then into the glass dishes (120 mm in diameter and 20 mm in depth) containing 100 mL of double distilled water (the blank), 5, 10, 50, 100 μmol L^{-1} $CdCl_2$ culture, respectively, for soaking. Then they were cultivated [28 ± 1 °C and 16:8 (L:D) hour photoperiod] in the constant temperature incubator for one day and every day from the second day to the fifth day, all the experimental processes and parameters were the same as those of experiment on the first day. All results were repeated for three times. At least five replicates were performed for each of the four $CdCl_2$ concentrations tested, plus control treatments (double distilled water). The root, bud, endosperm, which were collected from these glass dishes, were washed with 5 mmol L^{-1} pH 5.6 EDTA solution to remove the adhesion of Cd^{2+}. Finally, to determine enzyme activity, these biological tissues were crushed into fine particles in a mortar for future use.

Determination of antioxidant enzymes (SOD, POD, CAT, APX) and the degree of membrane lipid peroxidation (MDA): The enzyme activity (unless otherwise indicated, the determination of enzyme activity was all carried out at room temperature 25 °C or so) was calculated using the following formula 1:

$$U = \frac{\Delta OD \times 10 \times 1000 \times 60}{m \times 2.8 \times 20 \times 30} \text{mmol min}^{-1} \quad (1)$$

where ΔOD is the change of absorbance, 10 is 10.00 mL crude enzyme liquid extracted from each gram of material, 1000 is the rate of conversion of mL into μL, 60 is 1 min into 60 s, m is weight of fresh corn seeding tissue, unit: g, 2.8 is the absorption coefficient, unit: mmol L^{-1} cm^{-1}, 20 is 20 μL enzyme liquid determined, 30 is the change in absorbance value every 30 s.

Membrane lipid peroxidatio content calculation was carried out using the following formula 2:

$$MDA = [6.452 \times (D_{532} - D_{600}) - 0.559 \times D_{450}] \times \frac{V_t}{V_s \times W} \text{mmol g}^{-1} \quad (2)$$

where V_t is the total volume of extraction solution, unit: mL, V_s is the volume of determination extraction solution, unit: mL, W is the fresh weight of sample.

Determination of SOD activity: Superoxide dismutase activity test was conducted according to the method of even the three benzene phenol oxidation determination[12]. About 0.5 g fresh sample were weighed into a cooled mortar where 5.00 mL of 50 mmol L^{-1} pH 8.2 *Tris*-HCl buffer, 0.1 g PVP and a little silica sand were added and then homogenated under the condition of ice bath. Finally, at 4 °C the mixture was centrifuged at 9000 rpm for 20 min and supernatant was taken out and enzyme activity measured. 9.50 mL of 50 mmol L^{-1} pH 8.2 Tris-HCl buffer (the control tube instead of 10 mmol L^{-1} HCl) that was heated for 20 min at 25 °C was mixed with 0.10 mL of enzyme solution to be measured and 0.50 mL of 45 mmol L^{-1} even the three benzene phenol solution which was preheated at 25 °C *in vitro*, stirred to ensure good mixing, poured into the cuvette with 1 cm optical path and then spectrum absorption at 325 nm was measured every 30 s, continuously done for 20 min. Throughout, the autoxidation rate should be controlled at 0.06~0.07 OD min^{-1}. Superoxide dismutase autoxidation rate and SOD activity could be calculated from the corresponding absorption.

Determination of POD activity: POD activity test was carried out according to the methods reported previously by Xu and Ye[13] and Arnow in 1937. About 0.5 g fresh sample were weighed into a cooled mortar where 5.00 mL of 50 mmol L^{-1} pH 8.7 boric acid buffer containing 5 mmol L^{-1} sodium bisulfite, 0.1 g PVP and a little silica sand were added and then homogenated in ice bath. Finally, the mixture was centrifuged at 9000 rpm for 10 min at 4 °C and supernatants were taken out and enzyme activity measured. After the supernatant was diluted 10 times with boric acid buffer, 2.00 mL of 0.1 mmol L^{-1} pH 5.4 acetate buffer was mixed with 1.00 mL of 25 % guaiacol solution, 0.10 mL of enzyme solution and 0.10 mL of 0.75 % H_2O_2 solution which was preheated at 25 °C in cuvette with 1 cm optical path, stirred to ensure good mixing and then spectrum absorption at 460 nm was measured using interval reading method. The blank test was made in a similar manner.

Determination of catalase activity: Catalase activity was measured with UV-visible method, because H_2O_2 has an absorption peak at the wavelength of 294 nm whose intensity is proportional to the concentration of H_2O_2[14]. 0.10 mL of catalase extract was mixed with 2.00 mL of 30 μmol L^{-1} H_2O_2 and then diluted with double distilled water to 10.00 mL. The absorbance at 294 nm wavelength was determined by UV-visible spectrophotometer within 4 min after reaction, with 1.00 mL catalase enzyme extract mixture with 2.00 mL of double distilled water diluted to 10.00 mL again as blank control. Well linear relationship is found between the absorbance and the concentration of H_2O_2 in double distilled water only within the first 10 min after the reaction of catalase with H_2O_2, within 30 s after extract plus substrate the original absorbance data had to be recorded and then reaction results could be calculated.

Determination of APX activity: The reaction mixture was prepared according to Chaouia *et al.*[15]. 2 mL of sample tissue extract was mixed with 8.00 mL of 25 mmol L^{-1} pH 7.0 phosphate buffer, 0.50 mL of 0.5 mmol L^{-1} ascorbic acid, 2.00 mL of 2 mmol L^{-1} H_2O_2 and 0.50 mL of 0.1 mmol L^{-1} EDTA and the absorbance value decreased at 290 nm was detected (the extinction coefficient was 2.8 mmol L^{-1} cm^{-1}). The results were calculated based on the absorbance value decreased.

Determination of MDA activity: The sample under test were prepared using the procedures reported previously by Aravind *et al.*[16]. Both cleaned and dried sample (0.3000 g) were accurately weighed into a mortar where a little silica sand was added, powdered under liquid nitrogen. 8.00 mL of 25 mmol L^{-1} pH 7.0 phosphate buffer and then 0.5 % thiobarbituric acid (w/v) extract that had been dissolved in 20 % trichloroacetic acid (w/v) were added to the powder, followed by heating in a boiling water bath for 20 min (timing started as soon as the test tube solution appeared small bubbles). Then the reaction was over on the ice. Finally, the reaction solution was centrifuged at 9000 rpm for 10 min at room temperature. Supernatant was taken out and its volume measured. The absorbance at 450 nm, 532 nm and 600 nm was detected respectively and the extinction value at 532 nm, 600 nm and 450 nm measured using thiobarbituric acid solution as blank (the extinction coefficient was 155 mmol L^{-1} cm^{-1}). The content of MDA was determinated according to its absorbance value.

RESULTS AND DISCUSSION

Experiment results of SOD activity: The experiment results of SOD activity was represented in Fig. 1. It shows that SOD activity as a whole raised as the increase of Cd^{2+} concentration varied from 0 to 100 μmol L^{-1}. On the one hand, it arises rapidly with the increase of Cd^{2+} concentration in the range of 70~100 μmol $L^{-1}$and reaches a maximum at 100 μmol L^{-1}, which maintains at a higher level. On the other hand, with Cd^{2+} concentrations changing in the range of 10~70 μmol L^{-1}, it has little influence on SOD activity. Those indicate that there is little difference between the effects of different cadmium treatments on SOD activity of maize seeding and that of even the three benzene phenol autoxidation. The changes in UV-visible absorbency reflect the changes of concentrations of the substrate and the product. Therefore, the reaction rate is reflected indirectly in real time.

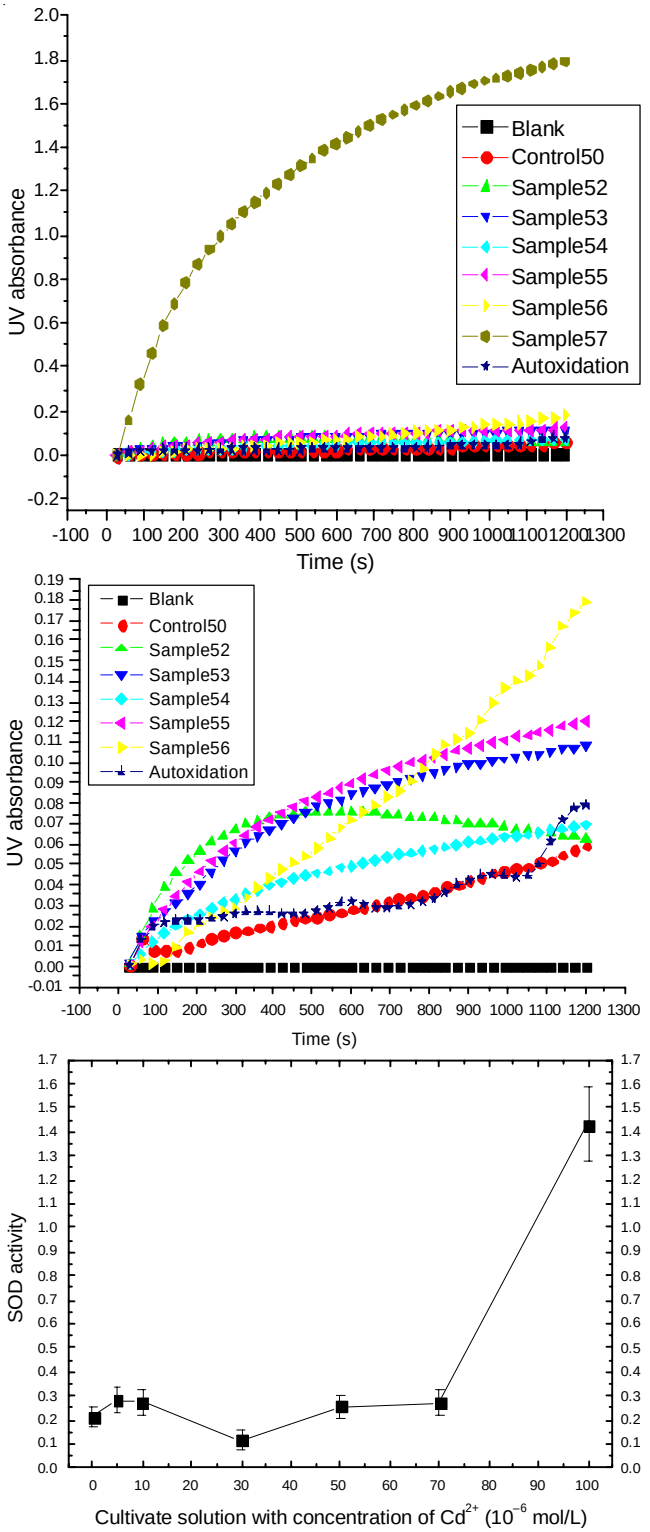


Fig. 1. Effects of different cadmium concentrations treatments on SOD activity of maize seeding

Experimental results of POD activity: The tests on the influence of cadmium contents on POD activity of maize seeding were conducted and the results were shown in Fig. 2. From Fig. 2, it is clear that POD activity rise swiftly with Cd^{2+} concentration increasing in the range of 70~100 μmol L^{-1} and arrives at a maximum at 100 μmol L^{-1}. However, Cd^{2+} concentrations in the 10~70 μmol L^{-1} range, POD activity overall is in low level, which is significantly higher than that of body blank.

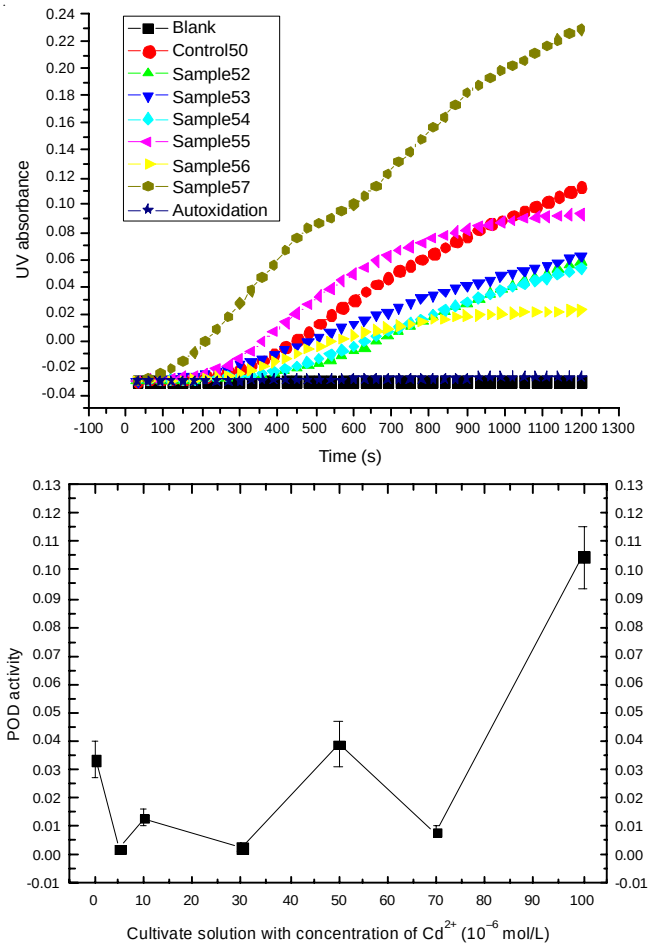


Fig. 2. Effects of different cadmium concentrations treatments on POD activity of maize seeding

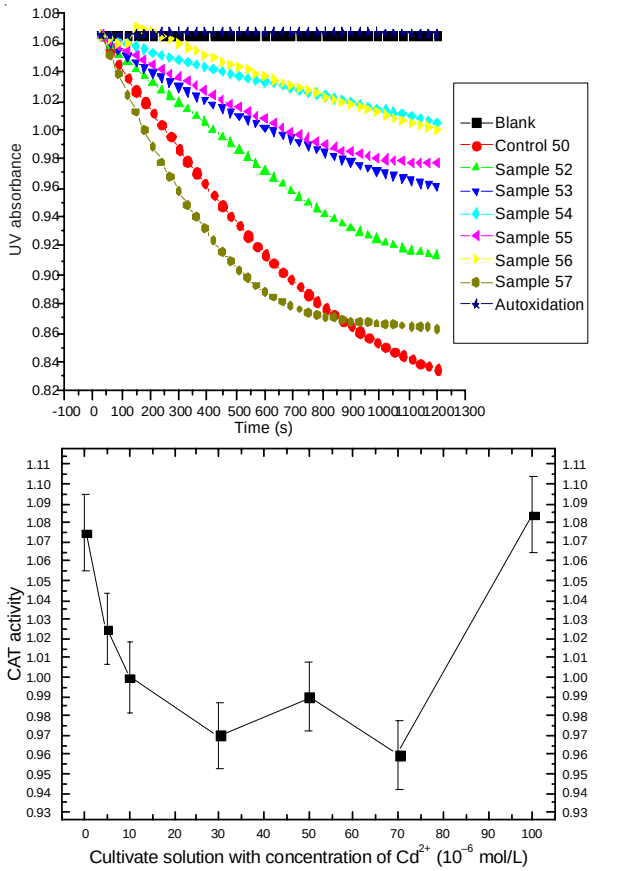


Fig. 3. Effects of different cadmium concentrations treatments on catalase activity of maize seeding

Experimental results of catalase activity: Fig. 3 shows the effect of different cadmium treatments on catalase activity of maize seeding. It can be seen that by and large, the Cd^{2+} concentration varied from 0 to 100 μmol L^{-1} exerted on catalase activity is characterized as complexity. Catalase activity decreases sharply with Cd^{2+} concentration increasing in the range of 0~10 μmol L^{-1}, while raises smartly in the range of 70~100 μmol L^{-1}. Moreover, the catalase activity generally maintains at a higher level in the concentrations above. However, Cd^{2+} concentrations in the 10~80 μmol L^{-1} range, catalase activity on the whole is in low level, which is higher than that of blank. On the other hand, it is also interesting to note that catalase activity for 100 μmol L^{-1} Cd^{2+} is almost equal to that on the absence of cadmium, whose values reach their maximum.

Experimental results of APX activity: The influence of cadmium contents on APX activity was investigated (Fig. 4). It can be seen from Fig. 4 that under Cd^{2+} stress, the content of MDA elevates swiftly with the increase of Cd^{2+} concentration from 70 to 100 μmol L^{-1} and arrives at a maximum at 100 μmol L^{-1}, while it is nearly equivalent and keeps at lower level basically in Cd^{2+} concentrations rang from 0 to 70 μmol L^{-1}. However, all are higher than those of blank group.

Experimental results of MDA activity: Effects of different cadmium contents on MDA concentration inside maize seeding were shown in Fig. 5. As can be seen, change trend of MDA content in maize seeding is not obvious with concentration of Cd^{2+} in

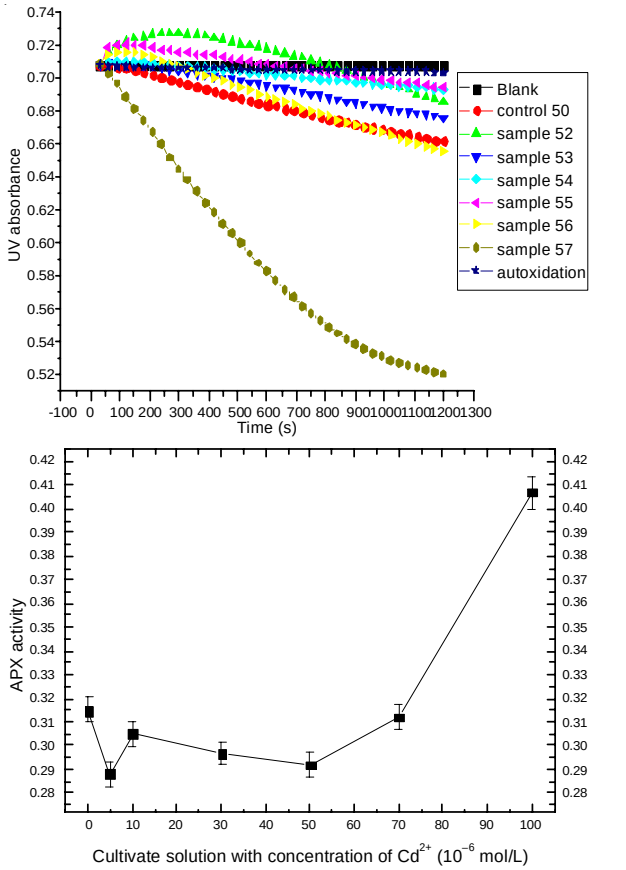


Fig. 4. Effects of different cadmium concentrations treatments on APX activity of maize seeding

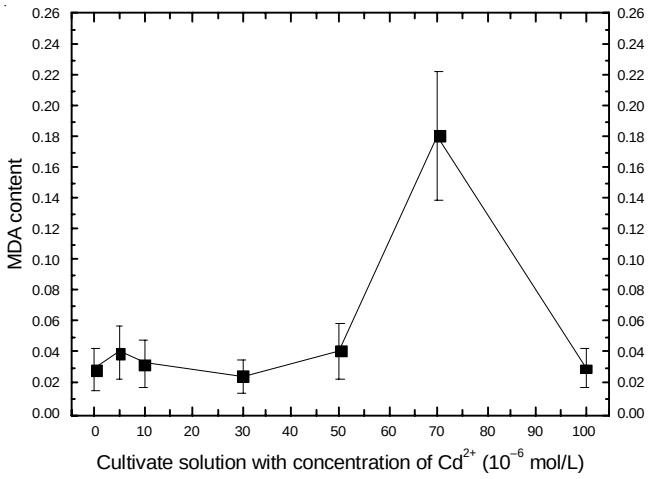


Fig. 5. Effects of different cadmium concentrations treatments on MDA concentration of maize seeding

the range of 0~50 μmol L^{-1}, while with concentration of Cd^{2+} in the range of 50~100 μmol L^{-1}, the total changing trend of MDA content is first increased dramatically and then decreased rapidly and the maximum MDA content obtained when the concentration of Cd^{2+} becomes 70 μmol L^{-1}.

Under the middle or high Cd^{2+} content of 50 or 100 μmol L^{-1} stress conditions, maize seeding have higher ability of accumulating Cd^{2+} and appears to demonstrate potential to resist toxicity when the highest dose accumulation of Cd^{2+} becomes 20 μmol g^{-1}; as the concentrations of Cd^{2+} are 5, 10, 30 or 50 μmol L^{-1}, there are no significant changes for normal permeability of cell membrane, therefore maize has a certain ability of tolerance to cadmium. Protective enzymes such as SOD, POD, CAT and APX in maize can make physiological responses at different degree which shows that anti-oxidation enzyme system of maize plant has a inherent capability of self-adjustment under the middle or high dose of Cd^{2+} stress, resulting in reducing the harm to cell membranes.

In order to explore the tolerance mechanism of maize and the antioxidant effects of the above-mentioned enzymes under cadmium stress conditions, the active centers of those enzymes were analyzed. The structural information about related enzymes was quoted from the Protein Data Bank (PDB). Even if the enzymes listed in the paper didn't come from maize, there were some values for reference to the experimental results. Superoxide dismutase, POD, CAT and APX are all using metal as the active centers of enzymes. For SOD, the centre metal ions are Cu^{2+} and Zn^{2+}, surrounded by the peptide chain, but unlike SOD, CAT, APX and POD are complex chelate of metal element Fe with porphyrin, which are complex protein with relatively stable structure. Compared with APX and POD, CAT has a high molecular weight which is about 4~12 times higher than that of APX or POD, as shown in Fig. 6.

Due to active sites, Cu^{2+} and Zn^{2+}, *via* weaker metal bond, coordination bond and hydrogen bond *etc*. to peptide chains, SOD molecules are more susceptible to environmental factors such as pH value, heavy metals, salinity, *etc*. Hence it is likely that Cu^{2+} and Zn^{2+} will be replaced by Cd^{2+} which is also a transition-metal and its structure is similar to that of Cu^{2+} or Zn^{2+} when a large number of heavy metals exist in cytoplasm, causing changes in the enzyme activity (chemical equation 1). The enzyme activity has certain tolerant ability to Cd^{2+} within a certain scope of cadmium, once beyond the tolerance threshold, the enzymes are inactivated rapidly. It should be stressed that the denaturalization means not only losing its activity in the narrow sense but also increasing or decreasing activity many times suddenly, namely there is very remarkable difference of enzyme activity between the normal cells and the ones impaired by Cd^{2+}. Fig. 1 determinates that when the concentration of Cd^{2+} goes more than 70 μmol L^{-1}, those enzymes are deactivated soon. The changes of normal SOD enzyme and denatured SOD enzyme and their relationship were represented using chemical equation 1.

$$\text{SOD(peptide chain - } Cu^{2+}, Zn^{2+}) \xrightleftharpoons{\text{pH, } Cd^{2+} \textit{ etc.}, \text{ salt } \textit{etc.}}$$

Normal SOD enzyme

$$\text{SOD (peptide chain - } Cd^{2+}) + Cu^{2+}, Zn^{2+}$$

Denatured SOD enzyme

Chemical equation 1: The changes of normal SOD enzyme and denatured SOD enzyme and their relationship

In contrast to the molecular structure of SOD, CAT, APX and POD are all complex chelate of metal element Fe with porphyrin, which are complex protein with relatively stable structure. Therefore, they are not vulnerable to environmental factors such as pH value, heavy metals, salinity, *etc*. (chemical equation 2). In addition, for CAT, with higher molecular weight, peptide chains around the molecule tends to protect well centre metal ions, making it more stable in extreme environment, which becomes verified by experimental results of enzyme activity, too. Chemical equation 2 shows the changes of normal POD, CAT and APX enzymes and denatured POD, CAT and APX enzymes and their relationship

$$\text{POD, CAT, APX (porphyrin - } Fe^{2+}) \xrightleftharpoons{\text{pH, } Cd^{2+} \textit{ etc.}, \text{ salt } \textit{etc.}}$$

Normal POD, CAT, and APX enzyme

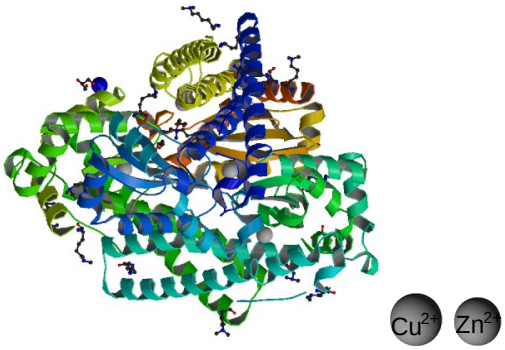
$$\text{POD, CAT, APX (porphyrin - } Cd^{2+}) + Fe^{2+}$$

Denatured POD, CAT, and APX enzyme

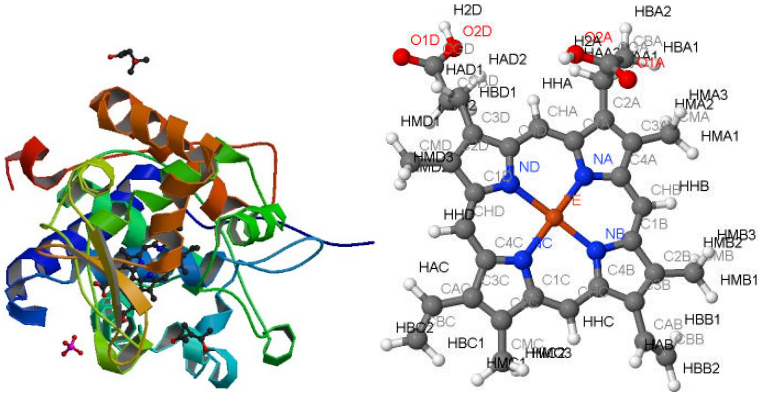
Chemical equation 2: The changes of normal POD, CAT and APX enzymes and denatured POD, CAT and APX enzymes and their relationship

Results of MDA experiments demonstrate that as Cd^{2+} content is below 70 μmol L^{-1}, plasma membrane is not basically destroyed by reactive oxygen under oxidative stress, so there still exists certain physiological functions. In other words, the level of membrane lipid peroxidation is lower. But when Cd^{2+} content is either above 70 μmol L^{-1} or under extreme conditions, the MDA content is higher, which makes plasma membrane destroyed seriously. That is to say that the content of active oxygen such as hydrogen peroxide, superoxide anion and so on produced in metabolic processes has a higher level, which is highly toxic to the organism especially the plasma membrane. Those results mentioned above provided another evidence for objective evaluation system of antioxidant enzyme.

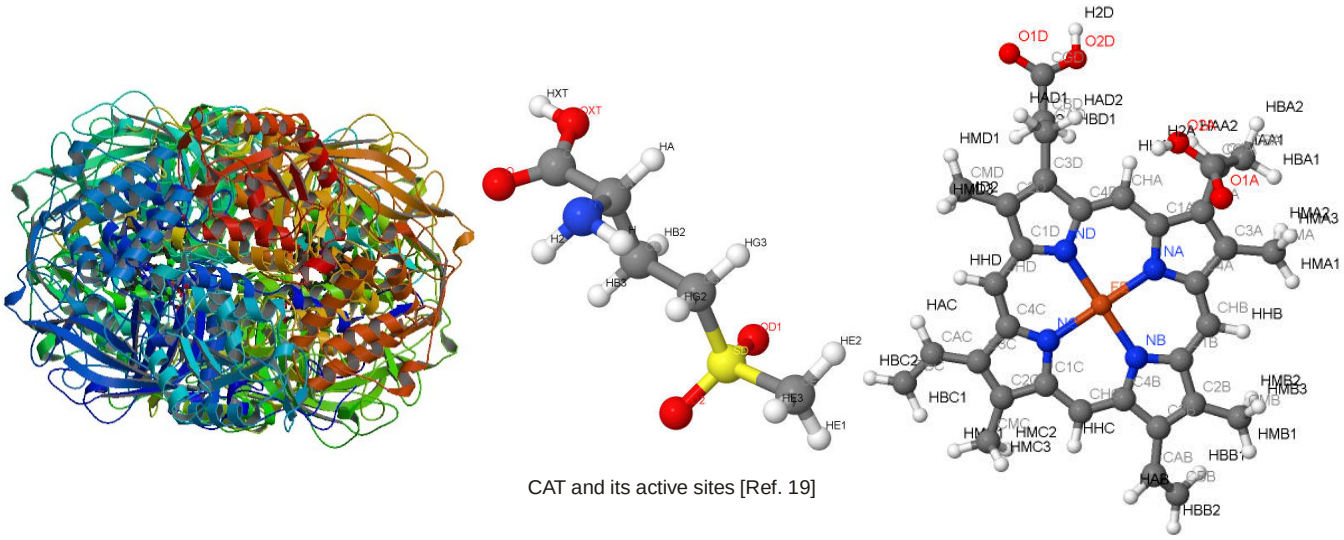
Many antioxidant enzymes in plant cells can reduce or eliminate the damage of ROS. Among them, SOD can convert



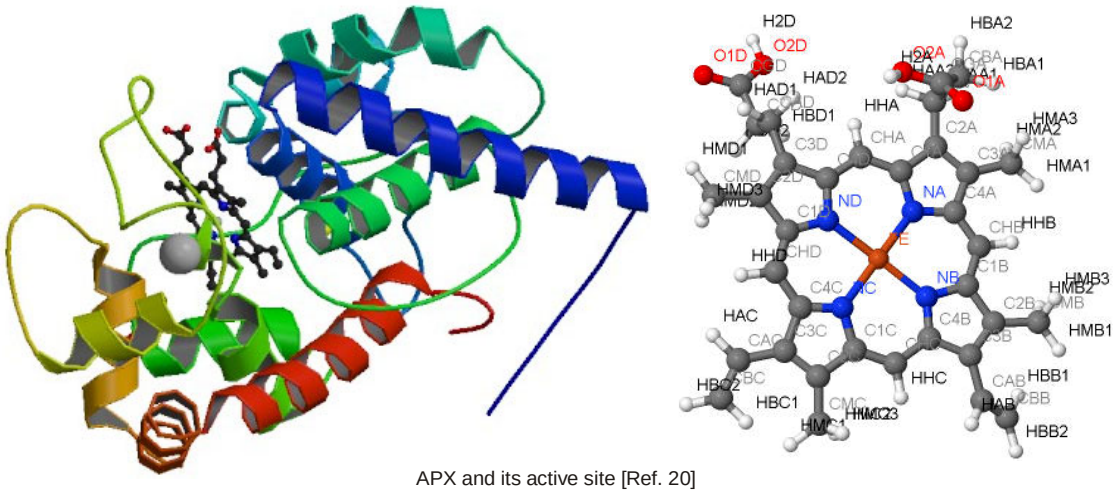
SOD and its active sites [Ref. 17]



POD and its active site [Ref. 18]



CAT and its active sites [Ref. 19]



APX and its active site [Ref. 20]

Fig. 6. Antioxidant enzymes (SOD, POD, CAT, APX) structure and active sites; Molecular weight: MW_{SOD} = 94774.72 Da; MW_{POD} = 34519.42 Da; MW_{CAT} = 347048.56 Da; MW_{APX} = 27804.31 Da

$O_2^{\bullet}$ into H_2O_2 and APX, GPX, POD, *etc.* translate further it into H_2O[21]. When the content of heavy metals is too high, in other words, it would exceed the tolerance detoxification and antioxidant capacity of that system, the imbalances of redox occur in a cellular system, leading to accumulation of more H_2O_2 in cells, which would cause significant damage to the cell membrane. For instance, after *Pinus sylvestris*[22] was treated by the solution containing 50 μmol L^{-1} Cd^{2+} for 6 h, SOD activity increased in its body, at the same time the activity of APX, *etc*. was restrained, which could contribute to the accumulation of H_2O_2 in the cell, inducing cell damage. The results of experiments indicate that the antioxidant enzyme activities of maize plumule treated by Cd^{2+} are not only changing in different degrees but the level of the enzyme activities also varying from different enzymes. That is, under cadmium stress, maize has strong physiological response and many antioxidant enzymes are involved in plant defense responses against cadmium synergistically. This result is

consistent with previous studies on plant such as *Artemisia willow*[23], *Salix acmophylla*[24], peas[15], Kidney beans[21], *etc*.

Conclusions

In a word, the above experimental results showed that various dosages of cadmium could lead to different damages to anti-oxidation enzyme system during the germination of cadmium-tolerant maize:

Firstly, during the germination of cadmium-tolerant maize, antioxidant enzyme system has been found to act effectively as oxygen free radical scavenger in a certain range of Cd^{2+} concentration, while Cd^{2+} concentration goes slightly beyond the threshold most antioxidant enzymes are easy to loss activities.

Secondly, the plasma membrane is severely damaged by MDA when the content of active oxygen such as hydrogen peroxide, superoxide anion, *etc*. produced in metabolic processes has a higher level, which is highly toxic to the organism, especially the plasma membrane.

Finally, those experimental results have also verified the information about the molecular structures of 4 antioxidant enzymes given above.

To solve the above-mentioned problem of plant poisoning caused by heavy metal, the key to success lies in looking for the suitable heavy metal detoxification reagent in subsequent work so that the damage of heavy metal stress to the antioxidant enzyme activities in plants can be lessened or even eliminated.

ACKNOWLEDGEMENTS

This work was financially supported by Anhui Province College Excellent Young Talents Fundation (No. 2013SQRL-051ZD), Higher Education in Anhui Province Provincial Revitalization Plan (No. 2014zytz014), Anhui Engineering Technology Research Center of Biochemical Pharmaceutical Foundation (No. BYEC1301), Anhui Natural Science Foundation (No. 1308085QB24) and the National College Students' Innovation and Entrepreneurship Training Plan (No. 201410367014), Anhui Research Project (No. KJ2015B037by).

REFERENCES

1. G.J.G. Pereira, S.M.G. Molina, P.J. Lea and R.A. Azevedo, *Plant Soil*, **239**, 123 (2002).
2. M.C. Romero-Puertas, F.J. Corpas, M. Rodriguez-Serrano, M. Gómez, L.A. del Río and L.M. Sandalio, *J. Plant Physiol.*, **164**, 1346 (2007).
3. L.M. Sandalio, H.C. Dalurzo, M. Gomez, M.C. Romero-Puertas and L.A. del Río, *J. Exp. Bot.*, **52**, 2115 (2001).
4. M.C. Romero-Puertas, M. Rodriguez-Serrano, F.J. Corpas, M. Gomez, L.A. Del Rio and L.M. Sandalio, *Plant Cell Environ.*, **27**, 1122 (2004).
5. K. Smeets, A. Cuypers, A. Lambrechts, B. Semane, P. Hoet, A. Van Laere and J. Vangronsveld, *Plant Physiol. Biochem.*, **43**, 437 (2005).
6. F.B. Wu, G.P. Zhang and P. Dominy, *Environ. Exp. Bot.*, **50**, 67 (2003).
7. V. Dixit, V. Pandey and R. Shyam, *J. Exp. Bot.*, **52**, 1101 (2001).
8. P.J. Kliebenstein, R.A. Monde and R.L. Last, *Plant Physiol.*, **118**, 637 (1998).
9. F.J. Corpas, A. Fernandez-Ocana, A. Carreras, R. Valderrama, F. Luque, F.J. Esteban, M. Rodríguez-Serrano, M. Chaki, J.R. Pedrajas, L.M. Sandalio, L.A. del Río and J.B. Barroso, *Plant Cell Physiol.*, **47**, 984 (2006).
10. C.H. Foyer, P. Descourvieres and K.J. Kunert, *Plant Cell Environ.*, **17**, 507 (1994).
11. H.Y. Zhang, Y.N. Jiang, Z.Y. He and M. Ma, *J. Plant Physiol.*, **162**, 977 (2005).
12. W.H. Xie, J.F. Yao and Q.S. Yuan, *Pharm. Ind. China*, **19**, 217 (1988).
13. L.L. Xu and M.B. Ye, *J. Nanjing Agric. Univ.*, **12**, 82 (1989).
14. R.L. Zheng, Free Radical Biology, Higher Education Press, Beijing, pp. 101-108 (1992).
15. A. Chaoui, S. Mazhoudi, M.H. Ghorbal and E. El Ferjani, *Plant Sci.*, **127**, 139 (1997).
16. P. Aravind and M.N.V. Prasad, *Plant Physiol. Biochem.*, **41**, 391 (2003).
17. Y. Sheng, D. Cascio and J.S. Valentine (2011); doi:10.2210/pdb3lsu/pdb.
18. A. Gumiero, M.P. Blakeley and C.L. Metcalfe (2011); doi:10.2210/pdb2ycg/pdb.
19. V. Jha, S. Louis and P. Chelikani (2011); doi:10.2210/pdb3p9p/pdb.
20. A. Gumiero, C.L. Metcalfe and A. Pearson (2011); doi:10.2210/pdb2xi6/pdb.
21. V. Dixit, V. Pandey and R. Shyam, *J. Exp. Bot.*, **52**, 1101 (2001).
22. A. Schutzendubel, P. Schwanz, T. Teichmann, K. Gross, R. Langenfeld-Heyser, D.L. Godbold and A. Polle, *Plant Physiol.*, **127**, 887 (2001).
23. T. Landberg and M. Greger, *J. Plant Physiol.*, **159**, 69 (2002).
24. M.B. Ali, P. Vajpayee, R.D. Tripathi, U.N. Rai, S.N. Singh and S.P. Singh, *Environ. Contam. Toxicol.*, **70**, 462 (2003).